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Short communication

Using glomalin as an indicator for arbuscular mycorrhizal hyphal growth: an example from a tropical rain forest soil

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Abstract

Glomalin concentrations of extra-radical arbuscular mycorrhizal (AM) hyphae were estimated by deploying hyphal in-growth cores containing glomalin-free sand in field soils in a tropical forest and in pot cultures. In field soils, glomalin was 0.044 ± 0.013 μg m^{-1} hyphae. In pot cultures glomalin concentrations were lower (range 0.0068–0.036 μg m^{-1}), and varied significantly among species. Using this technique, preliminary estimates of extraradical AM hyphal production on Inceptisols were 1.91 Mg $ha^{-1}yr^{-1}$ and on Oxisol were 1.47 Mg ha^{-1} yr^{-1}, but they could range between 0.9–5.7 Mg ha^{-1} yr^{-1}. These rates of hyphal production are approximately 10% (range 5–33%) of estimated above ground primary production of the forest.

Keywords: Hyphal production; Extra-radical hyphae; In-growth cores; *Acaulospora morrowiae*; *Glomus etunicatum*; *Glomus intraradices*; *Gigaspora rosea*

Biomass of arbuscular mycorrhizal (AM) fungi in soils and roots are commonly estimated based on hyphal lengths in soils and %colonization of fine roots. Hyphal-length estimates involve large uncertainties due to spatial variability, difficulty in extracting hyphae from soil aggregates, length-to-biomass conversion factors, and observer subjectivity (Stahl et al., 1995). Biochemical approaches for estimating fungal biomass have also proven problematic; ergosterol and chitin are not unique to AM fungi, and concentrations of AM-specific fatty acids in fungal biomass are highly variable and under strong environmental control (Wallender et al., 2001).

Here we assessed the use of the AM-fungal glycoprotein, glomalin (Wright et al., 1996; Wright and Upadhyaya, 1996) to quantify production of AM fungi in soil. Two advantages of glomalin as a bio-indicator for fungal production are that it is AM-fungus specific (extracts from many species of AM fungi and from many common soil pathogens and saprobes; Wright et al., 1996, Wright, unpublished data) and it is easily assayed (Wright and Upadhyaya, 1999; Wright et al., 1999). Uncolonized plant roots lack glomalin, and root glomalin increases with time (Wright et al., 1996) and hyphal colonization (Wright, unpublished data). Glomalin's ^{1}H NMR spectrum is different from that of humic acids (Nichols, 2003). However, as with ergosterol and fatty acids, the proportion of glomalin in fungal hyphae can be affected by environmental factors (Rillig and Steinberg, 2002) and can vary among fungal species and isolates (Wright et al., 1996). Here we used multiple approaches to assess whether glomalin accumulation can be used as a bio-indicator for hyphal growth.

We used hyphal in-growth cores (19.2 cm^3, packed with glomalin-free sand) to estimate glomalin production and to develop preliminary relationships between hyphal length and glomalin concentrations in a tropical forest soil at the La Selva Biological Station, Costa Rica. Our PVC cores were capped with 20 μm mesh to allow entry of hyphae but not roots, and were buried horizontally in the soil at 10 cm depth at preselected grid points in 12 0.5-ha forest plots (Clark and Clark, 2000). This provided samples in La Selva's two major edaphic types: Inceptisol terraces and Oxisol plateaus (Sollins et al., 1994). For intact cores (3–8/plot) we extracted hyphae by flotation, vacuum-trapped the hyphae on micropore membranes (2 μm), stained the hyphae with Direct Blue, and estimated hyphal length (Hanssen et al., 1974; Abbott et al., 1984). Glomalin was extracted from membranes in 20 mM citrate at 121 °C

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Table 1
Relationship between mean immunoreactive glomalin concentrations (μg) and mean hyphal length (m) for arbuscular mycorrhizal fungi extracted from sand in-growth cores from the La Selva Reserve, NE Costa Rica ($N = 30$), and from pot cultures using corn as a host plant

	Media	Yield (μg/m)	Yield [a] (μg/mg)	Yield[b] (μg/mg)
La Selva	Sand in-growth cores deployed in the field[c]	0.044 ± 0.013	10.8 ± 3.0	16.1 ± 4.5
Laboratory				
Acaulospora morrowiae	Corn grown on sand and in-growth cores	0.036 ± 0.013	8.8 ± 0.3.2	13.1 ± 4.8
Glomus etunicatum		0.022 ± 0.007	5.4 ± 1.8	8.1 ± 2.7
Glomus intraradices		0.0068 ± 0.0022	1.7 ± 0.5	2.5 ± 0.8
Gigaspora rosea		0.026 ± 0.009	6.3 ± 2.1	9.4 ± 3.2
Mean for all species		0.023 ± 0.005	5.5 ± 1.1	8.3 ± 1.6

[a] Calculated using data from Miller et al. (1995), Table 2 where 1 m hyphae = 4.12 μg dry weight.
[b] Stahl et al., 1995, 1 m of hyphae is 2.75 μg, assuming hyphal of diameter 3 μm density of 1.3 g cm^{-3} and solids content of 0.3 g g^{-1}.
[c] Glomalin-free sand was prepared through multiple extractions of glomalin from commercially available river sand using methods of Wright and Upadhyaya, 1996.

for 1 h (Wright and Upadhyaya, 1996), and assayed using a quantitative enzyme-linked immunosorbent assay (ELISA, Wright and Upadhyaya, 1999). Sand from cores also was extracted with citrate, and glomalin quantified by ELISA. Glomalin production in a core was the sum of hyphal plus sand ELISA values.

The mean of the ratio of immunoreactive (IR) glomalin to AM hyphal length extracted from the in-growth cores was 0.044 ± 0.013 μg glomalin m^{-1} ($N = 30$ cores, Table 1). Using two estimates of specific hyphal weight (4.12 μg m^{-1}, Miller et al., 1995; and 2.75 μg m^{-1}, Stahl et al., 1995) we estimate that glomalin production is 10.8 ± 3.0 and 16.1 ± 4.5 μg mg^{-1} of the production of AM fungal hyphal dry mass, respectively (Table 1). We did not find a significant linear relationship between the hyphal length and the glomalin produced in the cores, but found variability in the ratio of glomalin to hyphal length (CV = 28%). Similar CV's have been found for hyphal lengths in other studies (Stahl et al., 1995). Larger CV's of mean hyphal biomass have been observed using fatty acid methods (Olsson and Wilhelmsson, 2000).

We compared the La Selva estimates to those from four fungal cultures at USDA, Maryland. Contents of the entire core were extracted with citrate for glomalin yield prior to measuring hyphal length. Preliminary tests found no significant loss of hyphae during extraction of glomalin. Immunoreactive glomalin yields per hyphal length were lower in the pot cultures than the field soils, and there were significant differences in yields among fungal species (ANOVA, $F_{3,59} = 3.945$, $P = 0.013$, Table 1). Using two estimates of specific hyphal length, IR glomalin yield varied across species from 1.7 to 8.8 μg mg^{-1} (mean 3.6 ± 0.9 μg mg^{-1}), or 2.5 to 13.1 μg mg^{-1} (mean 5.8 ± 1.1 μg mg^{-1}). *Acaulospora morrowiae*, a common species at La Selva (Lovelock et al., 2003), had the highest glomalin yield.

Yields of IR glomalin exponentially declined with increasing hyphal length (Fig. 1). This could be due to: (1) increasing errors in hyphal length estimates with greater amounts of hyphae, including errors in hyphal identification with the possible inclusion of saprotrophic fungi, or (2) the greater abundance of fine hyphae where greater hyphal lengths were observed; finer hyphae may have lower concentrations of glomalin.

For two species we determined the relationship between hyphal biomass and extracted glomalin by weighing dried hyphae before extraction. IR glomalin yield was significantly lower (ANOVA, $F_{1,9} = 5.95$, $P = 0.0374$) for *Gigaspora rosea* (6.4 ± 0.7 μg mg^{-1}, $N = 6$) compared to *Glomus etunicatum* (16.7 ± 4.6 μg mg^{-1}, $N = 5$). Overall, mean yields of hyphal-associated IR glomalin, whether estimated from field data or measured in the laboratory using direct measurements or a conversion factor for hyphal mass per length, ranged from 3.6 to 16.7 μg mg^{-1} hyphal biomass. Variability in yield could be due to environmental effects (e.g. variation in soil chemical and physical

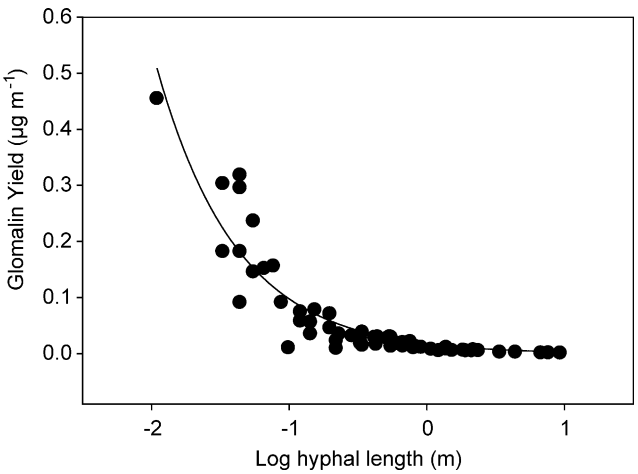

Fig. 1. Relationship between immunoreactive glomalin yield (μg m^{-1} hyphae) and observed hyphal length for arbuscular mycorrhizal fungi extracted from pot cultures using corn as a host plant. Regression line of best fit is $Y = a \exp(-bX)$, where a is 0.0175 and $b$is 1.72, $R^2 = 0.85$.

Table 2
Glomalin accumulation after 4 weeks of deployment of sand in-growth cores over two soil types with differing fertility (Inceptisol, $N = 27$; Oxisol, $N = 37$) within the primary forest of the La Selva Reserve in NE Costa Rica

	Inceptisol	Oxisol	Prairie
Glomalin (mg in 4 weeks)	0.0300 ± 0.0030	0.0231 ± 0.0029	
Estimated hyphal length ($m\ cm^{-3}\ d^{-1}$)	1.27 ± 0.13	0.98 ± 0.12	0.22
Hyphal production ($Mg\ ha^{-1}\ yr^{-1}$)	1.91 ± 0.19	1.47 ± 0.18	0.31

Growth of hyphae in length is then calculated on the basis of 0.044 μg glomalin/m hyphae. Hyphal biomass is estimated as 4.12 $\mu g\ m^{-1}$ (Miller et al., 1995). Annual peak rate of hyphal production estimated by Miller et al., 1995 in prairie and pasture soils are given for comparison.

characteristics) on fungal metabolism, as well as due to differences among fungal species.

Preliminary estimates of AM hyphal production using glomalin accumulation were conducted by placing glomalin-free in-growth cores in forest plots that spanned the soil fertility gradient at La Selva (Lovelock et al., 2004). After 4 weeks there was greater IR glomalin accumulation in the more fertile Inceptisols than in the less fertile Oxisols (Table 2, ANOVA, $F_{1,53} = 6.89$, $P = 0.027$). Based on these data and a mean production of IR glomalin of 0.044 $\mu g\ m^{-1}$ hyphae, we estimate AM hyphal production in the top 10 cm of soil on the two soil types are: Inceptisols, 1.91 $Mg\ ha^{-1}\ yr^{-1}$; Oxisols (less fertile), 1.47 $Mg\ ha^{-1}\ yr^{-1}$. These values are higher than the peak annual hyphal production rates of 0.31 $Mg\ ha^{-1}\ yr^{-1}$ estimated for North American prairie (Miller et al., 1995) and sand dune vegetation (0.28 $Mg\ ha^{-1}\ yr^{-1}$, Olsson and Wilhelmsson, 2000), and are similar to estimates from pot cultures (0.78–1.20 $Mg\ ha^{-1}\ yr^{-1}$, Abbott and Robson, 1985).

Using the 3.6–16.7 $\mu g\ mg^{-1}$ range of observed mean IR-glomalin yields, we estimate the upper and lower bounds for extraradical AM hyphal production in the top 10 cm of forest soil at La Selva: Inceptisols, 1.2– 5.7 $Mg\ ha^{-1} yr^{-1}$; Oxisols, 0.9–4.4 $Mg\ ha^{-1}\ yr^{-1}$. If supported by longer-term measurements, such values indicate that extraradical AM hyphal production could range between 5 and 33% of estimated aboveground net primary production at La Selva (17 $Mg\ ha^{-1}\ yr^{-1}$; Clark and Clark, unpublished data). Additional, as yet unquantified components of AM production, are the production of hyphae within roots, hyphae in roots or soils below 10 cm depth, losses to belowground consumers, and the production of spores. In addition to the importance of AM fungi in the maintenance of soil structure (Jastrow and Miller, 1997; Wright and Upadhyaya, 1998; Rillig et al., 1999), AM fungi may play a large role in carbon cycling, especially if their turnover time is rapid, as recent ^{14}C experiments have indicated (Staddon et al., 2003). Additional experimentation testing the effects of environment on yields of glomalin and on transformations of glomalin in soils, in addition to improved descriptions of the morphology and architecture of AM hyphal networks, would greatly enhance the confidence in using short-term accumulation of glomalin as an indicator for hyphal production in soils.

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